

AMENDMENTS

In the specification:

On page 6, please delete the paragraph on page 6, line 24, to page 7, line 5, and substitute therefor:

Figure 11 shows assays demonstrating the interaction of BAG-family proteins with Hsc70/ATPase. ~~(A) Two~~ Figure 11A shows two-hybrid assays using yeast expressing the indicated fusion proteins. Blue color indicates a positive interaction, resulting in activation of the lacZ reporter gene. ~~(B) In~~ Figure 11B shows *in vitro* protein assays using GST-fusion proteins and ³⁵S-labeled *in vitro* translated proteins. ~~(C) Co~~ Figure 11C shows co-immunoprecipitation assays using anti-FlagTM or IgG1 control antibodies and lysates from 293T cells expressing FlagTM-tagged BAG-1 (beginning at residue 116 of SEQ ID NO:2), BAG-2 (SEQ ID NO:4), BAG-3 (SEQ ID NO:6), Daxx, or Apaf-1.

On page 14, please delete the paragraph on lines 1-15, and substitute therefor:

Similar minor modifications may also include amino acids deletions or insertions or both. Guidance in determining which amino acid residues may be modified as indicated above without abolishing the desired biological functionality may be determined using computer programs well known in the art, for example, DNASTARTM software. In addition, the derivative may also result from chemical modifications to the encoded polypeptide, including but not limited to the following, replacement of hydrogen by an alkyl, acyl, or amino group; esterification of a carboxyl group with a suitable alkyl or aryl moiety; alkylation of a hydroxyl group to form an ether derivative. Further a derivative may also result from the substitution of a L-configuration amino acid with its corresponding D-configuration counterpart.

On page 30, please delete the paragraph on page 30, line 29, to page 31, line 16, and substitute therefor:

Cells were recovered and incubated with 0.5 mg/ml lysozyme in 50 mM Tris (pH 8.0), 150 mM NaCl, 1% TweenTM-20, 0.1% 2-mercaptoethanol, 5 mM EDTA, 1 mM PMSF and a mixture of other protease inhibitors obtained from Boehringer Mannheim (1697498) at room temperature for 0.5 h, followed by sonication. Cellular debris were pelleted by centrifugation at 27,500 g for 10 min and the resulting supernatants were incubated with 30 ml of glutathione-SepharoseTM (Pharmacia) at 4°C overnight. The resin was then washed with 20 mM Tris (pH 8.0), 150 mM NaCl, 0.1% TweenTM-20, and 0.1% 2-mercaptoethanol until the OD 280 nm reached <0.01. For removal of GST, the resin with immobilized GST-fusion protein was incubated with 10U of thrombin (Boehringer, Inc.) at 4°C in 20 mM Tris (pH 8.0), 150 mM NaCl, 0.1% TweenTM-20, 0.1% 2-Mercaptoethanol, and 2.5 mM CaCl₂ overnight. Released proteins were then purified on Mono QTM (HR10/10, Pharmacia) by FPLC using a linear gradient of 0.5M NaCl at pH 8.0 and dialyzed into chaperone assay buffer.

On page 31, please delete the paragraph on page 31, line 17, to page 32, line 4, and substitute therefor:

The ability of BAG-2 (SEQ ID NO:4) or BAG-3 (SEQ ID NO:6) to bind Hsc70/ATPase in solution was then examined. GST control or GST-BAG proteins were immobilized on glutathione-SepharoseTM and tested for binding to ³⁵S-labeled *in vitro* translated (IVT) proteins. Immunoprecipitation and *in vitro* GST-protein binding assays were performed as described by Takayama et al., *supra* (1997), using pCI-Neo flag FlagTM or pcDNATM3-HA into which human Bag-2 (clone #11) or human BAG-3 (clone #28) had been subcloned for *in vitro* translation of ³⁵S-L-methionine labeled proteins or expression in 293T cells. As shown in Figure 11B, ³⁵S-Hsc70/ATPase bound *in vitro* to GST-BAG-1, GST-BAG-2, and GST-BAG-3 but not to GST-BAG-1 (ΔC) or several other control proteins. BAG-1 (beginning at residue 116 of SEQ ID NO:2), BAG-2 (SEQ ID NO:4), and BAG-3 (SEQ ID NO:6) also exhibited little or no binding to themselves or to each other, demonstrating that these proteins do not strongly homo- or hetero-

dimerize or oligomerize. It should be noted, however, that BAG-2 (SEQ ID NO:4) displayed weak interactions with itself in binding assays and produced a positive result in yeast two-hybrid experiments, demonstrating that it can have the ability to self-associate.

On page 32, please delete the paragraph on page 32, lines 6-23, and substitute therefor:

The ability of BAG-2 (SEQ ID NO:4) and BAG-3 (SEQ ID NO:6) proteins to interact in cells with Hsc70 was tested by expressing these proteins with N-terminal FlagTM epitope tags in 293T human epithelial cells using co-immunoprecipitation assays as described previously (Takayama et al., supra (1997)). cDNAs encoding the λ -phage cloned regions of BAG-2 and BAG-3 were subcloned in-frame into pcDNATM3-FlagTM. Anti-FlagTM immune complexes prepared from 293T cells after transfection with plasmids encoding FlagTM-BAG-1, FlagTM-BAG-2, or FlagTM-BAG-3 were analyzed by SDS-PAGE/immunoblot assay. As shown in Figure 10C, antiserum specific to Hsc70 detected the presence of BAG proteins associated with Hsc70, whereas control immune-complexes prepared with IgG1 as well as anti-FlagTM immune complexes prepared from cells transfected with FlagTM-tagged control proteins, Daxx and Apaf-1, did not contain Hsc70 associated protein. These results further demonstrate that BAG-family proteins specifically bind to Hsc70.

On page 32, please delete the section header on lines 24-25 and substitute therefor:

C. BIAcoreTM Assay of BAG Protein Binding to the ATPase Domain of Hsc70

On page 32, please delete the paragraph on page 32, line 26, to page 33, line 4, and substitute therefor:

BAG-1 (beginning at residue 116 of SEQ ID NO:2) is known to bind tightly to the ATPase domain of Hsc70 (Stuart et al., *J. Biol. Chem.*, In Press (1998)). BAG-2 (SEQ ID NO:4) and BAG-3 (SEQ ID NO:6) proteins were therefore, examined for their ability to bind to Hsc70/ATPase. The affinity and binding kinetics of BAG-2 (SEQ ID NO:4) and BAG-3 (SEQ ID NO:6) to Hsc70/ATPase was also compared to that of BAG-1 (beginning at residue 116 of SEQ ID NO:2) for Hsc70/ATPase, using a surface plasmon resonance technique (BIAcoreTM) which has been described previously (Stuart et al., *supra*, (1998) which is incorporated herein by reference).

On page 33, please delete the paragraph on page 33, line 5, to page 34, line 8, and substitute therefor:

BAG-family proteins were produced in bacteria and purified to near homogeneity as shown in Figure 12A and described above in Example I. The purified BAG-1 (beginning at residue 116 of SEQ ID NO:2), -2 (SEQ ID NO:4), and -3 (SEQ ID NO:6) proteins were then immobilized on biosensor chips and tested for their interactions with Hsc70 in the soluble phase. Kinetic measurements were performed using a BIAcoreTM-II instrument with CM5 sensor chip and Amine Coupling Kit (Pharmacia Biosensor AB, Sweden). Briefly, for immobilization of proteins, the sensor chip was equilibrated with HK buffer (10 mM Hepes (pH 7.4), 150 mM KCL) at 5 µl/min, then activated by injecting 17 µl of 0.2M N-ethyl-N'-(3-diethylaminopropyl)-carbodiimide and 0.05M N-hydroxysuccinimide (NHS/EDC) followed by 35 µl of the protein of interest, in 10 mM acetate, pH 3.5-4.5. Excess NHS-ester on the surface was deactivated with 17 µl 1M ethanolamine-HCL (pH 8.5). After immobilization, 5 µl of regeneration buffer (50 mM phosphate (pH 6.8) and 4M GuHCl) was injected. For binding assays, Hsp70 (Sigma, H8778) was dissolved in HK buffer, and injected at 10 µl/min across the prepared surface at various concentrations. The surface was regenerated after each injection with 5 µl of regeneration buffer. The rate constants K_{ass} and K_{diss} were generated with BIAevaluation ~~software~~ software

3.01 (Pharmacia Biosensor AB). Addition of Hsc70 to chips containing BAG-1 (beginning at residue 116 of SEQ ID NO:2), BAG-2 (SEQ ID NO:4) or BAG-3 (SEQ ID NO:6) resulted in concentration-dependent binding, as reflected by an increase in the Response Units (RU) measured at the chip surface (shown in Figure 3B). In contrast, Hsc70 failed to display interactions in BIAcoreTM assays with a variety of control proteins as well as a mutant of BAG-1 lacking a C-terminal portion of the BAG domain which is required for Hsc70-binding (Figure 3B). Furthermore, flowing of various control proteins such as GST, BSA and Bcl-XL over the BAG-1 (beginning at residue 116 of SEQ ID NO:2), BAG-2 (SEQ ID NO:4), or BAG-3 (SEQ ID NO:6) chips resulted in negligible interaction. These results further demonstrate the specificity with which BAG-family proteins interact with and bind to Hsc70.

On page 36, please delete the paragraph on lines 14-30 and substitute therefor:

In an additional refolding assay, described previously by Minami et al., *J. Biol. Chem.* 271:19617-24, 1996), purified Hsc70 and human DnaJ homolog Hdj-1 (Hsp 40) were used with additional cofactors provided in reticulocyte lysates (5% v:v) to produce a system capable of refolding denatured luciferase. Briefly, additional cofactors included, recombinant Luciferase (Promega: QuantiLumTM), that had been heat denatured at 42°C for 10 min, 1.8 µM Hsc70 (Sigma; purified from bovine brain), 0.9 µM Hsp40, and various recombinant purified proteins. Luciferase activity was measured (Promega luciferase assay kit) using a luminometer (EG&G Berthold, MicroLumat luminometer, Model #LB96P). All results were normalized relative to non-denatured luciferase that had been subjected to the same conditions. Control reactions lacking ATP, Hsc70, or Hsp40 resulted in negligible luciferase refolding.

On page 37, please delete the paragraph on page 37, line 22, to page 38, line 5, and substitute therefor:

Hip was purified as His₆-protein. The fusion protein was induced from pET28-Hip (V. Prapapanich et al., *Mol Cell Biol.*, 18:944-952, 1998, which is incorporated herein by reference) with 0.1 mM IPTG at 25°C for 6 h in BL21 cells. Cells from 1L of culture were resuspended into 50 ml of 50 mM Phosphate buffer (pH 6.8), 150 mM NaCl, and 1% (v/v) TweenTM-20 and then incubated with 0.5 mg/ml lysozyme at 25°C for 0.5 h, followed by sonication. After centrifugation at 27,500 g for 10 min, the resulting supernatant was mixed with 15 ml nickel resin (Qiagen, Inc.) at 4°C for 3 h with 25 mM imidazol. The resin was then washed with 50 mM phosphate buffer (pH 6.8), 25 mM imidazol, 150 mM NaCl and 0.1% TweenTM-20 until the OD₂₈₀ nm reached a value of <0.01. His₆-Hip protein was eluted with 250 mM imidazol in washing buffer (Qiagene, Inc.) and purified on Mono QTM (HR10/10 Pharmacia) by FPLC using a linear gradient of 0.5M NaCl at pH 8.0, followed by dialysis in chaperone assay buffer.